

WE CLAIM:

1. A composition for stimulating an immune system, said composition comprising a plurality of fused cells, each of which fused cells is generated by fusion between at least one mammalian dendritic cell and at least one mammalian non-dendritic cell that expresses a cell-surface antigen, wherein at least half of the fused cells express, in an amount effective to stimulate an immune system, (a) a MHC class II molecule, (b) B7, and (C) the cell-surface antigen.

2. The composition of claim 1, wherein the mammalian non-dendritic cell is a cancer cell.

3. The composition of claim 1, wherein the mammalian dendritic cell and the mammalian non-dendritic cell are obtained from the same individual.

4. The composition of claim 3, wherein the individual is a human.

5. The composition of claim 4, wherein the cell-surface antigen is a cancer antigen.

6. The composition of claim 1, wherein the mammalian dendritic cell and the mammalian non-dendritic cell are obtained from different individuals of the same species.

7. The composition of claim 6, wherein the species is *Homo sapiens*.

8. The composition of claim 7, wherein the cell-surface antigen is a cancer cell antigen.

9. A method of producing a fused cell useful for stimulating an immune system, comprising:

providing a first fused cell formed by fusion between at least one mammalian dendritic cell and at least one mammalian non-dendritic cell that expresses a cell-surface antigen; and

fusing the first fused cell with at least one mammalian dendritic cell to produce a second fused cell that is useful for stimulating an immune system.

10. The method of claim 9, wherein the second fused cell expresses (i) a MHC class II molecule, (ii) B7, and (iii) the cell-surface antigen.

11. The method of claim 9 wherein all of the mammalian dendritic cells and the mammalian non-dendritic cells are human cells.

12. The method of claim 11, wherein the cell surface antigen is a cancer antigen.

13. A method of producing a fused cell, comprising:  
providing a cell sample comprising (i) a first plurality of mammalian dendritic cells, and  
(ii) a plurality of mammalian non-dendritic cells expressing a cell-surface antigen; and  
contacting the cell sample with a fusion agent to produce a post-fusion population of cells comprising a fused cell that is the fusion product of at least one of the dendritic cells and at least one of the non-dendritic cells.

14. The method of claim 13, wherein the fused cell is useful for stimulating an immune system.

15. The method of claim 14, wherein the fused cell expresses (a) a MHC class II molecule, (b) B7, and (C) the cell-surface antigen.

16. The method of claim 13, wherein the mammalian dendritic cells are cultured from (i) bone marrow cells, or (ii) peripheral blood cells.

17. The method of claim 16, wherein the time between the contacting step and the separating step is less than 10 days.

18. The method of claim 13, further comprising fusing the isolated fused cell with at least one mammalian dendritic cell to produce a secondary fused cell.

19. The method of claim 18, wherein the secondary fused cell expresses (i) a MHC class II molecule, (ii) B7, and (iii) the cell-surface antigen.

20. The method of claim 19, wherein all of the mammalian dendritic cells and the mammalian non-dendritic cells are human cells.

21. The method of claim 20, wherein the cell surface antigen is a cancer antigen.

22. A method of stimulating the immune system in an individual, said method comprising administering the composition of claim 1 to the individual.

23. The method of claim 22, wherein the individual has a condition selected from the group consisting of:

susceptibility to infection with an intracellular pathogen;

infection with an intracellular pathogen;

cancer; and

predisposition to develop cancer.

24. A method of stimulating the immune system in a human, said method comprising administering the composition of claim 1 to the human.

25. The method of claim 24, wherein the mammalian dendritic cells are obtained from the human or an identical twin of the human.

26. The method of claim 25, wherein the non-dendritic cells are cancer cells obtained from the human.

27. The method of claim 25, wherein the cell-surface antigen is a cancer antigen.

28. The method of claim 25, wherein the cell-surface antigen is an antigen derived from a pathogen.

29. The method of claim 28, wherein the pathogen is a virus.

30. The method of claim 27, wherein the cancer antigen is MUC1.

31. The method of claim 30, wherein the individual has one of the following conditions or predisposition to develop one of the following conditions: breast cancer, ovarian cancer, pancreatic cancer, prostate gland cancer, lung cancer and myeloma.

32. A substantially pure population of educated, antigen-specific immune effector cells expanded in culture at the expense of hybrid cells, wherein the hybrid cells comprise antigen presenting cells fused to cells that express one or more antigens.

33. The population according to claim 32, wherein the antigen presenting cells are dendritic cells.

34. The population according to claim 32, wherein the cells expressing the antigen(s) are tumor-specific.

35. The population according to claim 32, wherein the antigen-specific immune effector cells are cytotoxic T lymphocytes.

36. The population according to claim 32, wherein the antigen-specific immune effector cells are genetically modified cells.

37. The population according to claim 32, wherein the hybrid cells are genetically modified cells.

38. The population according to claim 36, wherein the genetic modification comprises introduction of a polynucleotide.

39. The population according to claim 38, wherein the polynucleotide encodes a peptide, a ribozyme or an antisense sequence.

40. The population according to claim 32, wherein the antigen presenting cells and the cells that express one or more antigens are autologous.

41. The population according to claim 32, wherein the antigen presenting cells and the cells that express one or more antigens are allogeneic.

42. A substantially pure population of educated, antigen-specific immune effector cells produced by culturing immune effector cells with hybrid cells, wherein the hybrid cells are antigen presenting cells fused to cells that express one or more antigens and wherein the educated, antigen-specific immune effector cells are expanded at the expense of the hybrid cells.

43. The population according to claim 42, wherein the antigen presenting cells are dendritic cells.

44. The population according to claim 42, wherein the cells recognizing the antigen(s) are tumor-specific.

45. The population according to claim 42, wherein the antigen-specific immune effector cells are cytotoxic T lymphocytes.

46. The population according to claim 42, wherein the antigen-specific immune effector cells are genetically modified cells.

47. The population according to claim 42, wherein the hybrid cells are genetically modified cells.

48. The population according to claim 46, wherein the genetic modification comprises introduction of a polynucleotide.

49. The population according to claim 48, wherein the polynucleotide encodes a peptide, a ribozyme or an antisense sequence.

50. The population according to claim 42, wherein the antigen presenting cells and the cells that express one or more antigens are autologous.

51. The population according to claim 42, wherein the antigen presenting cells and the cells that express one or more antigens are allogeneic.

52. The population according to claim 42, wherein the immune effector cells are

naïve.

53. The population according to claim 42, wherein the immune effector cells are educated.

54. The population according to claim 42, wherein the immune effector cells are produced by culturing immune effector cells with hybrid cells in the presence of a cytokine.

55. The population of claim 54, wherein the cytokine is IL-2.

56. A method for producing antigen-specific immune effector cells comprising culturing immune effector cells in an effective amount of hybrid cells, wherein the hybrid cells comprise antigen presenting cells fused to cells expressing one or more antigens and wherein the antigen-specific immune effector cells are produced at the expense of the hybrid cells.

57. The method according to claim 56, wherein the antigen presenting cells are dendritic cells.

58. The method according to claim 56, wherein the dendritic cells are derived from blood, bone marrow or skin and the immune effector cells are derived from tumor tissue.

59. The method according to claim 56, wherein the cells expressing the antigen(s) and the immune effector cells have been enriched from a tumor.

60. The method according to claim 56, wherein the antigen presenting cells and the cells that express one or more antigens are autologous.

61. The method according to claim 56, wherein the antigen presenting cells and the cells that express one or more antigens are allogeneic.

62. The method according to claim 56, wherein the immune effector cells are naïve.

63. The method according to claim 56, wherein the immune effector cells are

educated.

64. The method according to claim 56, further comprising culturing the immune effector cells in the presence of an effective amount of cytokine.

5

65. The method according to claim 64, wherein the cytokine is IL2.

66. A method of adoptive immunotherapy, comprising administering to a subject a population of educated, antigen-specific immune effector cells expanded in culture at the expense of hybrid cells, wherein the hybrid cells comprise antigen presenting cells fused to cells that express one or more antigens.

10

67. A method of adoptive immunotherapy comprising administering to a subject a population of educated, antigen-specific immune effector cells made by culturing naïve immune effete cells with hybrid cells, wherein the hybrid cells are antigen presenting cells fused to cells that express one or more antigens and wherein the educated, antigen-specific immune effector cells are expanded at the expense of the hybrid cells.

15  
20  
25  
30  
35  
40  
45  
50  
55  
60  
65  
70  
75  
80  
85  
90  
95  
100

68. The method according to claim 66 or 67, wherein the antigen presenting cells are dendritic cells.

69. The method according to claim 66 or 67, wherein the dendritic cells are derived from blood, bone marrow or skin and the immune effector cells are derived from a tumor.

25

70. The method according to claim 66 or 67, wherein the cells that express one or more antigens and the immune effector cells have been enriched from a tumor.

71. The method according to claim 66 or 67, wherein the immune effector cells are cytotoxic T cells.

30

72. The method according to claim 66 or 67, wherein the antigen specific immune effector cells administered to the subject are allogeneic.

73. The method according to claim 66 or 67, wherein the antigen specific immune

effector cells administered to the subject are autologous.

74. The method according to claim 66 or 67, further comprising culturing the immune effector cells in the presence of an effective amount of a cytokine.

5

75. The method according to claim 74, wherein the cytokine is IL2.

76. A method of identifying a fragment of a gene encoding an antigen recognized by the population of antigen-specific immune effector cells according to claim 32 or 42, the method comprising:

10

(a) providing a population of first cells of claim 32 or 42, wherein the cells have an identified major histocompatibility complex (MHC) restriction and one or more second cells having a compatible major histocompatibility complex (MHC) to the first cell but which does not express antigen;

(b) identifying polynucleotides encoding a peptide sequence motif in the antigen displayed by the population of first cells of claim 32 or 42;

(c) identifying polynucleotides which are aberrantly expressed by the first cells as compared to one or more second cells; and

(d) comparing the polynucleotides identified in step (c) with the polynucleotides motifs identified in step (b) to identify the fragment of the gene encoding the antigen recognized by the immune effector cell.

15  
20  
25  
30  
35  
40  
45  
50  
55  
60  
65  
70  
75  
80  
85  
90  
95  
100  
105  
110  
115  
120  
125  
130  
135  
140  
145  
150  
155  
160  
165  
170  
175  
180  
185  
190  
195  
200  
205  
210  
215  
220  
225  
230  
235  
240  
245  
250  
255  
260  
265  
270  
275  
280  
285  
290  
295  
300  
305  
310  
315  
320  
325  
330  
335  
340  
345  
350  
355  
360  
365  
370  
375  
380  
385  
390  
395  
400  
405  
410  
415  
420  
425  
430  
435  
440  
445  
450  
455  
460  
465  
470  
475  
480  
485  
490  
495  
500  
505  
510  
515  
520  
525  
530  
535  
540  
545  
550  
555  
560  
565  
570  
575  
580  
585  
590  
595  
600  
605  
610  
615  
620  
625  
630  
635  
640  
645  
650  
655  
660  
665  
670  
675  
680  
685  
690  
695  
700  
705  
710  
715  
720  
725  
730  
735  
740  
745  
750  
755  
760  
765  
770  
775  
780  
785  
790  
795  
800  
805  
810  
815  
820  
825  
830  
835  
840  
845  
850  
855  
860  
865  
870  
875  
880  
885  
890  
895  
900  
905  
910  
915  
920  
925  
930  
935  
940  
945  
950  
955  
960  
965  
970  
975  
980  
985  
990  
995

77. The method of claim 76, wherein step (c) is performed prior to step (b).

25

78. A vaccine comprising an antigen identified according to the method of claim 76.

79. A method of identifying a polypeptide encoding a sequence motif present in an antigen recognized by the population of antigen-specific immune effector cells according to claim 32 or 42, comprising:

30

(a) providing a cell population of antigen-specific immune effector cells of claim 32 or 42 and having an identified major histocompatibility complex (MHC) restriction; and

(b) identifying a polypeptide encoding a sequence motif in the antigen recognized by the immune effector cells.



80. A vaccine comprising an antigen identified according to the method of claim 79.

81. A vaccine comprising a composition for stimulating an immune system according  
5 to claim 1 and a pharmaceutically acceptable carrier.

82. The vaccine of claim 81 further comprising an immunoregulatory cytokine.

83. A vaccine comprising a composition for stimulating an immune system produced  
10 according to the method of claim 9.

84. A vaccine comprising the population of antigen-specific immune effector cells  
of claim 32 or 42 and a pharmaceutically acceptable carrier.

85. A vaccine comprising antigen-specific immune effector cells produced according  
15 to the method of claim 56.

86. A method of identifying a fragment of a gene encoding an antigen recognized by  
the population of antigen-specific immune effector cells according to claim 32 or 42, the method  
20 comprising:

(a) providing a population of first cells of claim 32 or 42, wherein the cells  
have an identified major histocompatibility complex (MHC) restriction and one or more second  
cells having a compatible major histocompatibility complex (MHC) to the first cell but which  
does not express antigen;

25 (b) identifying polynucleotides encoding a peptide sequence motif in the  
antigen displayed by the population of first cells of claim 32 or 42;

(c) identifying polynucleotides which are aberrantly expressed by the first  
cells as compared to one or more second cells; and

(d) comparing the polynucleotides identified in step (c) with the  
30 polynucleotides motifs identified in step (b) to identify the fragment of the gene encoding the  
antigen recognized by the immune effector cell  
wherein the polynucleotides are identified using the SAGE method.

87. The method of claim 86, wherein step (c) is performed prior to step (b).

88. A method of identifying a polypeptide encoding a sequence motif present in an

antigen recognized by the population of antigen-specific immune effector cells according

5 to claim 32 or 42, comprising:

(a) providing a cell population of antigen-specific immune effector  
cells of claim 32 or 42 and having an identified major histocompatibility complex (MHC)  
restriction; and

(b) identifying a polypeptide encoding a sequence motif in the antigen  
10 recognized by the immune effector cells  
wherein the polypeptide is identified using the SPHERE method.